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Full Length Research Paper

Preventing of Ehrlich tumor metastasis in liver, kidney and spleen by electromagnetic field

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The aim of the present work is to study the therapeutic effects of 4.5 Hz of electromagnetic radiation as a preventive agent on the metastasis of Ehrlich tumor which occurred in some organs. The tumor was implanted intramuscularly in the left thigh of mice. Sixty female Balb/c mice were used, twenty as normal group and the other forty were divided equally into 2 groups namely A and B. Group A was the control while group B was whole body exposed to square amplitude modulated waves (QAMW) of frequency 4.5 Hz for a period of 40 h at a rate of 4 h/day starting day 11 post tumor implantation. At the end of the experiment liver, kidney and spleen were excised for all groups. Kidney and spleen were subjected to histological examination. Fragments of the freshly excised liver were subjected to histopathological examination and the other parts were used for dielectric measurements. The results of this work indicate that the treatment of Ehrlich tumor by QAMW caused a decrease in the percentage of metastasis, also there was a decrease in the values of relative permittivity, conductivity and dielectric loss of group B as compared with group A. The statistical methods and analysis for evaluation of the results were done by calculating arithmetic means and standard deviations for percentage of metastasis and dielectric measurements. It may be concluded that whole body exposure of the animals injected with Ehrlich tumor to 4.5 Hz QAMW can inhibit tumor growth in secondary sites or distant organs and can be used as preventive agent to murine metastatic development.

Key words: Electromagnetic field, liver, kidney, spleen, Ehrlich tumor metastasis, tissue dielectrics.

INTRODUCTION

There are a number of preventive cancer agents like radiotherapy, chemotherapy and hormonal therapy. However these agents form some risks and have long term treatments. In recent published work by our group Fadel et al (2005), we figured out the inhibiting effect of resonance frequency of 4.5 Hz square amplitude modulated waves (QAMW) on the aggressiveness and properties of Ehrlich tumor implanted in female mice. In that work, the implanted tumor in the thigh of the mice at day 10 post implantation was exposed to different frequencies of QAMW for a period of 10 h at a rate of 2 h/day. The wave carrier frequency was 10 MHz. It was found that exposure to 4.5 Hz QAMW stopped the aggressiveness of tumor growth and caused pronounced reduction of the telomerase activity in the tumor. Our main problem since that time was to find an answer for the question, “if treatment of the tumor in an organ (primary site) can work as a preventive agent of metastasis into the distant organs (secondary site)?

Some reports have demonstrated the antitumor effectiveness of electromagnetic field on organs. Turler et al. (1998) studied the effectiveness of low level direct current therapy in liver metastasis and the influence of polarity (anode or cathode in the center of the tumor) or current dose (60 or 80 coulomb/cm^3). It has been concluded in that research that direct current therapy may offer an alternative minimal invasive method in the

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treatment of liver metastasis. Robertson et al. (1998) have examined the effect of increasing current dose on the volume of the lesion induced in normal rat liver. Increasing the field strength by 10 times decreased the total volume of necrosis. Using an animal model with induced hepatic metastasis, have analyzed the effectiveness and the tumor growth dynamics after direct current application. Their results have confirmed the effectiveness of low level direct current application as a potential modality for the treatment of hepatic metastasis (Turler et al., 2000).

The lethal effect of extremely low frequency (ELF) pulsed electromagnetic field on human cancer cells has been studied by Traitcheva et al. (2003) The results have shown a decreasing vitality of human K-562 and U-937 cancer cells in suspension. Ciria et al. (2004) have indicated that electrotherapy with low level direct electric current is feasible and effective in the treatment of the Ehrlich and fibrosarcoma Sa-37 tumors. The sensitivity of these tumors to direct electric current (dc) and survival rates of mice depend on both the amount of electrical charge and the type of the tumor. A certain intensity of the field should be reached in order to get complete regression of the tumor. However, no dc current can pass in a dielectric medium such as liver tissue because its electrical impedance is infinity and the possible effect could be due to electrolysis that might occur at enough field strengths. All biological tissues have electrical properties of dielectric materials where the electrical impedance is inversely frequency (f) dependent and for dc current f is zero.

The effects of a rectified pulsed semi-sine signal magnetic field (15 mT amplitude, 120 pulses/s) have been studied by Cameron et al. (2005). Therapeutic electromagnetic field (TEMF) in mice bearing a human MDA MB231 breast cancer xenograft showed that TEMF had significant reducing effect on lung metastatic sites and slower tumor growth than did untreated mice. Richard et al.(2006) have discovered a new drug-free therapy for treating solid skin tumors, pulsed electric fields greater than 20 kV/cm with rise times of 30 ns and durations of 300 ns penetrate into the interior of tumor cells and cause tumor cell nuclei to rapidly shrink and tumor blood flow to stop. Therefore the present study was undertaken to assess whole body exposure to 4.5 Hz QAMW on animals carrying Ehrlich tumor implanted in the thigh as a preventive agent against tumor infiltrative growth and metastatic process into extra- thigh origin example, Liver, kidney and spleen.

MATERIALS AND METHODS

A preliminary experiment was done on 30 female Balb/c mice injected intramuscularly into the left flank with 0.2 ml of a suspension containing $1x10^6$ cells/ml isolated from murine Ehrlich ascites carcinoma in mice, prepared in the national cancer institute (NCI), Cairo University, Egypt. The animals were divided into 3 groups; they were sacrificed at day 10, 20 and 25 post implantation (PI). At the end of the experiments, histological sections of liver, kidney and spleen from each sacrificed animal were prepared to evaluate the presence of metastasis by using the histopathological criteria. The results of this experiment indicated that metastasis started to occur in the studied organs in 25% of the animals at day 10 PI and in 100% at day 25 PI. These results gave us the impetus to start animal treatment at day 11 PI in order to prevent metastasis occurrence.

In the target experiment sixty female Balb/c of average weight 20 ± 2 gm each were used .Twenty were kept as normal group (neither injected nor exposed) while the other 40 were injected with the same concentration as in the pilot experiment of the Ehrlich suspension and divided into 2 groups, group A is the control group while group B is whole body exposed to 4.5 Hz QAMW for a period of 40 h at rate of 4 h/day for 2 weeks, 5 days/week. After each exposure, the animals were returned back to their cages receiving the same environmental conditions (light, ventilation, temperature etc.) and the same diet as control.

At the end of the exposure period, animals of group B were divided into two subgroups, the first subgroup of 10 mice was sacrificed and the liver, kidney and spleen were removed for histopathological studies, the other subgroup of 10 mice was used for measuring the dielectric properties of liver. The same measurements were done at the same time for control group A. In Egypt there are no restrictions for the use of experimental animals for research studies.

Whole body treatment with electromagnetic field

In this experiment, animals were whole body exposed to electromagnetic waves, generated from 2 generators. The carrier wave was 10 MHz sine wave with amplitude ± 2 V_{pp} generated from a synthesized arbitrary waveform generator type Thurbly Thander Instruments (TTi TGA 1230) manufactured by Huntingdon Cams England .This wave was square amplitude modulated (AM) by a second wave generator model AFG 310 manufactured by Sony tectonics, Japan with a modulation depth of ±1 V_{pp}. The output of the second generator that carries QAMW was connected to two parallel copper mesh electrodes of separation distance 1.5 cm and area 180 cm^2. During the whole body exposure the animals were housed between the parallel capacitance electrodes as shown in Figure 1.

Dielectric measurements

In this study the dielectric measurements were made for the whole bulk of the liver for groups A and B. Dielectric measurements were also made to normal liver. The liver was immediately excised and placed between a pair of 1 cm diameter black platinum circular electrodes for dielectric measurements which began within 5 min in order to prevent dryness of the tissue. The sample between the electrode pair was maintained at a constant press, and the distance between the electrodes was measured while the liver sample was filling the whole volume between the electrodes through the use of a micrometer. Dielectric measurements were made in the range from 0.1 to 10 MHz using a loss factor meter type Hioki, 3532, LCR Hi TESTER, 1999, Japan. During measurements, the sample between the electrodes was kept at a constant temperature of 24 ± 0.1°C.

The capacitance of the tissue was measured at each frequency and the resistance was recorded, each run was taken 10 times and the average was considered. The relative permittivity of the sample was calculated for each frequency using the relation:

$$\varepsilon' = \frac{Cd}{\varepsilon_0 A} \qquad (1)$$

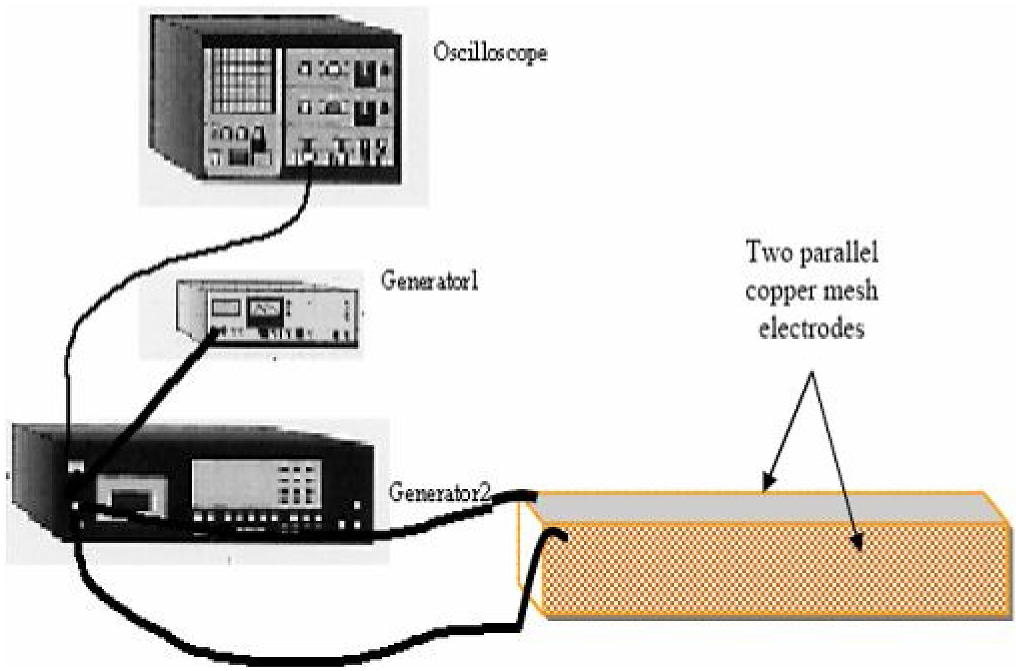

Figure 1. The electromagnetic field treatment system.

where d is the inter-electrode distance in meter, A area of electrode in m^2 measured from the cell used and ε_0 permittivity of free space.

The loss tangent (tan δ), the dielectric loss ε'' and the A.C. conductivity σ were calculated from the relations:

$$\tan \delta = \frac{\varepsilon''}{\varepsilon'} \quad (2)$$

$$\sigma = 2\pi f \varepsilon'' \varepsilon_0 \quad (3)$$

where, f is the frequency applied in Hz and R is the resistance of the specimen in Ohm. The value of the dielectric constant έ falls from high value ε'_s to ε'_∞ as the frequency increases through the dispersion region where έ is the real part of the complex permittivity. The dielectric dispersion ($\Delta\varepsilon'$) was calculated by applying the relation:

$$\Delta \varepsilon' = \varepsilon'_s - \varepsilon'_\infty \quad (4)$$

The relaxation time was calculated from the equation:

$$\tau = 1/2\pi f_c \quad (5)$$

Where f_c is the critical frequency corresponding to the midpoint of the dispersion curve. All the measurements had been done for animals of all groups, the average reading of five runs were used to calculate the means and standard deviations for each group. Student's t test was used to determine the significant differences between groups (Snedecor and Coechran, 1967).

Histopathological preparation

Fragments of liver, kidney and spleen were fixed in 10% formalin and processed by the paraffin method. Hematoxylin - Eosin stained sections were examined using Olympus optic microscope Cx31. As the study was concentrated on the liver as it showed high metastasis, the percentages of metastasis in liver for groups A and B at day 25 were measured. The histological sections were viewed and scanned through the use of a scanning and measuring image contour analyzer (SAMICA), type ELBEK, GmbH, Germany. The system is provided with an electronic camera connected to a computer through an interface built in card. The percentage of metastasis was evaluated from the percentage of the diseased region relative to the whole area of the section.

RESULTS

Dielectric data

The results showed metastasis in liver, kidney and spleen 10 days post implantation. In kidney and spleen, the metastasis was lower where almost any primary tumor site can deposit metastasis in the hepatic tissue. There is a scientific clamp where metastasis can occur when a malignant cell moves through the lymph and vascular systems (blood vessels) involved with the primary cancer site to the other organs (Venook, 1994) Therefore the experiment of dielectric measurements was focused on liver as a host area of metastasis. Figure 2 shows the

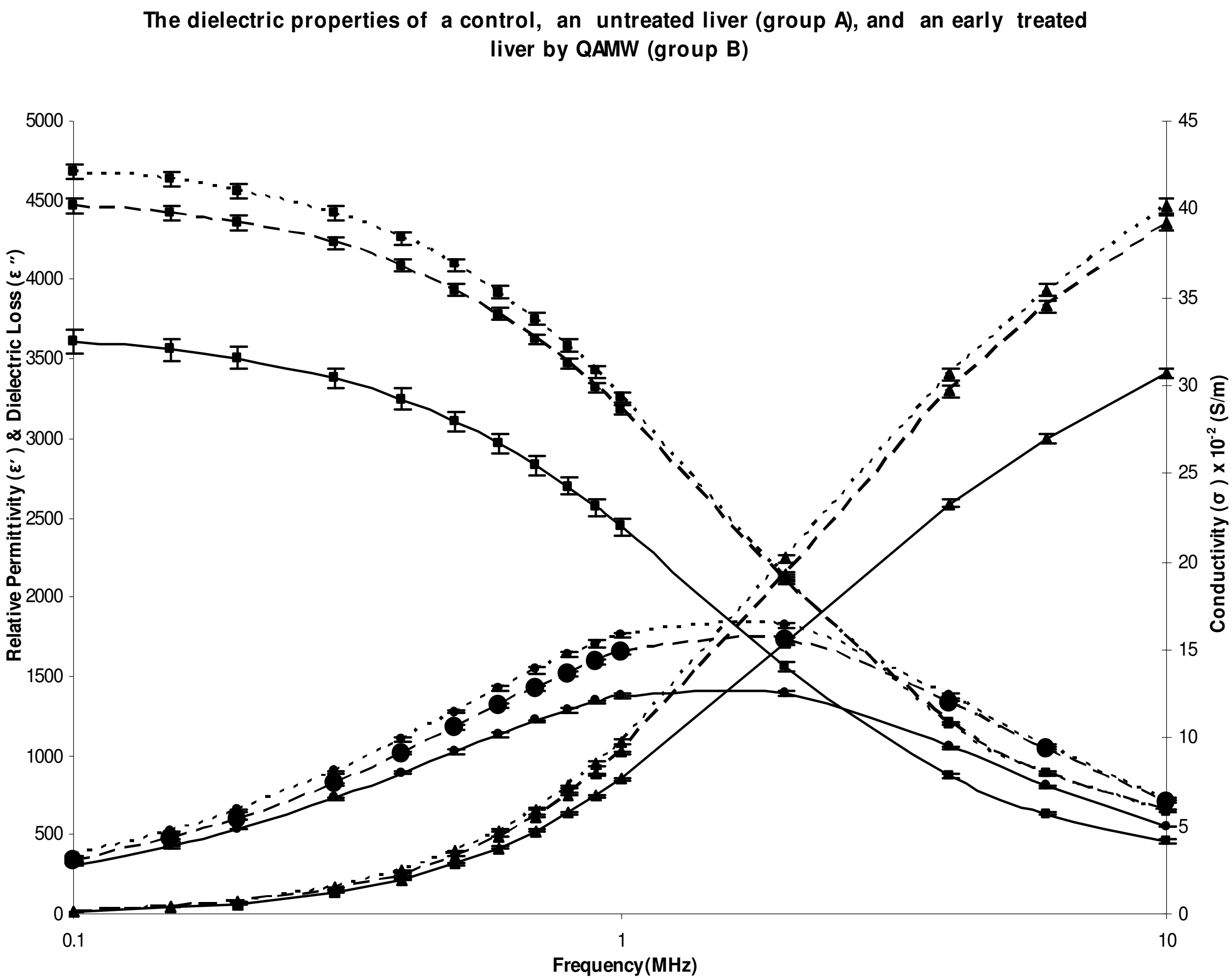

Figure 2. The variation of the relative permittivity ε′ (■), the dielectric loss ε ″ (●) and the electric conductivity σ (▲) as function of the frequency in the range of 0.1–10 MHz of normal liver(— line), an untreated liver from the group A (…….line), and early treated liver by 4.5 Hz QAMW from group B (------- line). (Note: The frequency is represented on log scale.

variation of the relative permittivity ε′ , the dielectric loss ε″ and the electric conductivity σ as function of the frequency in the range of 0.1-10 MHz of normal liver, an untreated liver from the group A , and earlier treated liver by 4.5 Hz QAMW from group B . It is clear from the figure, that the permittivity passed through a dielectric dispersion and the decrease in the values of permittivity was accompanied by an increase in the values of conductivity which we considered as indicating confidence in the measurements.

Histopathological appearance

Liver

Macroscopically, metastatic tumor in the liver appeared in the form of discrete nodularity. Nodules were yellowish enlarged filled with liquefied material. In the comparison with normal hepatic tissue (Figure 3a), liver of group A revealed high percentage of metastasis at day 25.

Table 1 shows the percentage of metastasis at day 25 for group A and group B. The t-test done for the data presented in Table 1 revealed that there was a high significant difference between animals of groups A and B($p \leq 0.01$).

Malignant darkly stained epithelial cells were tensely arranged in masses invading hepatocytes appeared as ground glass degeneration (Figure 3b). In the liver of group B metastatic aggregates sharply disappeared. Some scattered focal neoplastic cells were occasionally metastasized. Liver parenchyma was extensively occupied with hepatocytes. Apoptic cells were also observed (Figure 3c).

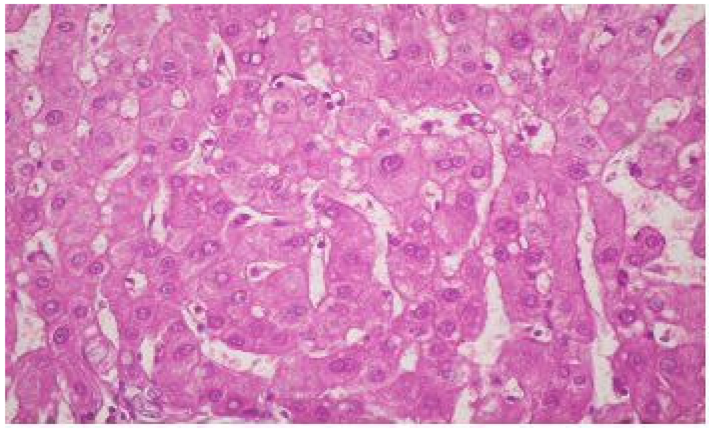

Figure 3a. A photographic cross section for a normal liver tissue (Magnification x 400), shows normal cells with single nuclei.

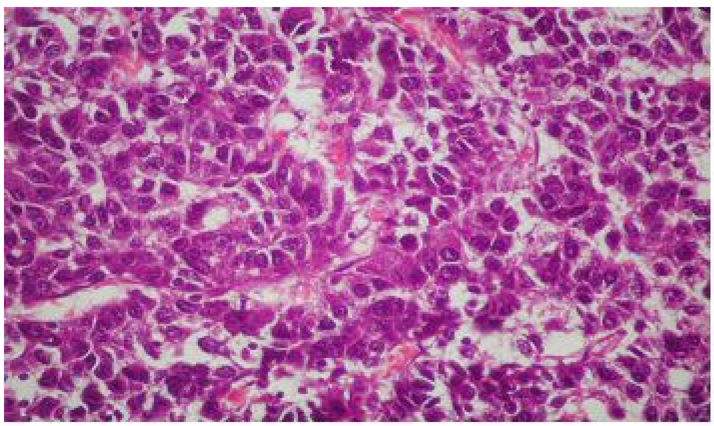

Figure 3b. Liver of group A revealed high grade of metastasis at day 25. Malignant darkly stained epithelial cells were tensly arranged in masses invading hepatocytes appeared as ground glass degeneration (Magnification x 400).

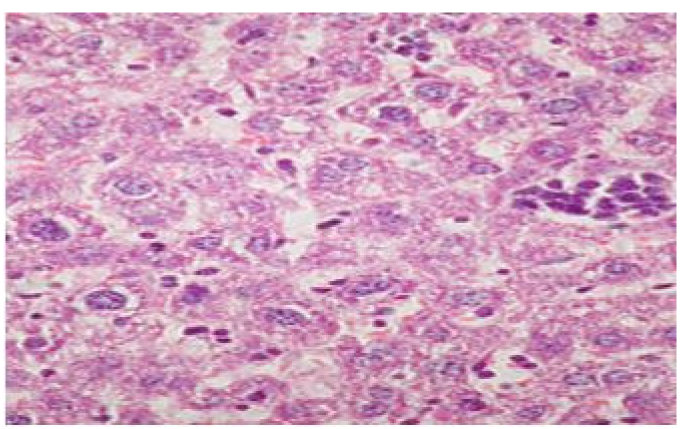

Figure 3c. A photographic cross section for a liver of group B showing some scattered focal neoplastic cells were occasionly metastasized. Liver parenchyma was extensively occupied with hepatocytes. Apoptic cells were also observed. (Magnification x 400).

Table 1. Shows the percentage of metastasis for liver tissue at day 25 for groups A and B.

Parameter	Group A	Group B	Level of significance
Percentage of metastasis (**%**)	39.17% ± 2.1	19.4% ± 1.5	high

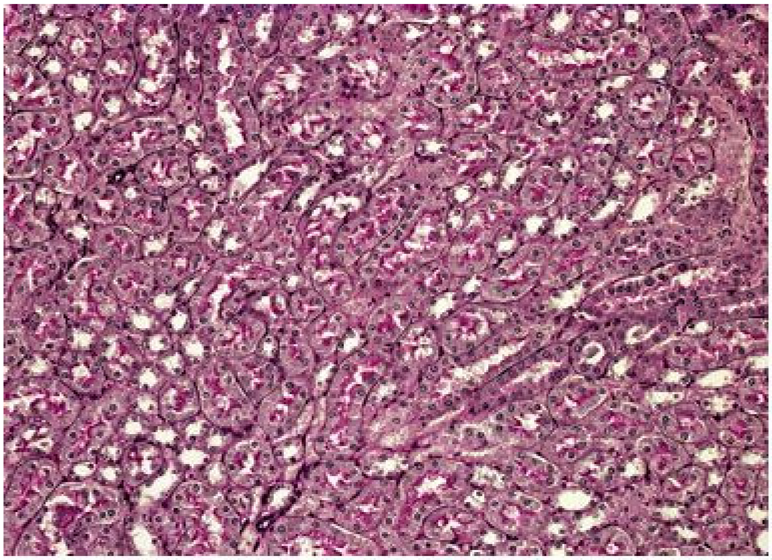

Figure 4a. A photographic cross section for a normal kidney tissue shows normal cells with single nuclei (Magnification x 200).

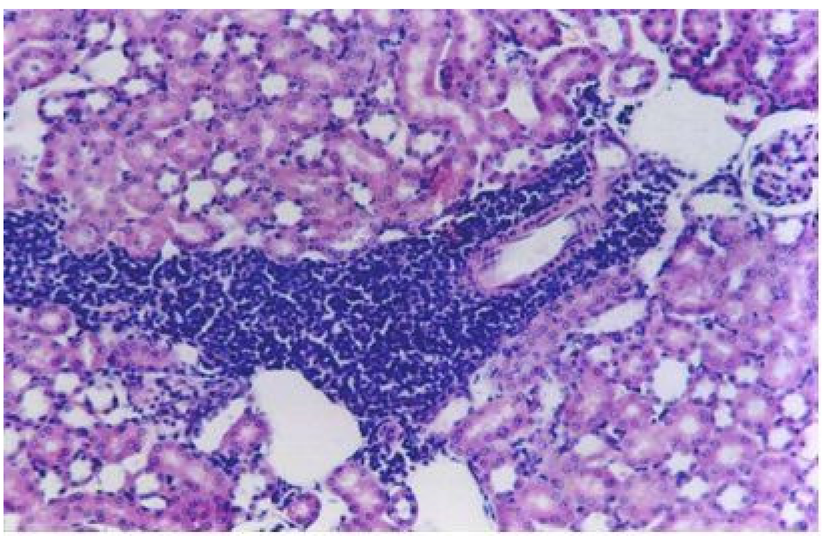

Figure 4b. A histological section of the kidney of the animals from group A shows a metastasized Ehrlich neoplastic cells were heavily accumulated in renal tissue, Tumorigenic mass containing darkly stained and densely packed cells were arranged around or near blood vessels (Magnification x 200).

Kidney

In group A, metastasized Ehrlich neoplastic cells were heavily accumulated in renal tissue. In the comparison with normal renal tissue (Figure 4a), tumorigenic mass containing darkly stained and densely packed cells were arranged around or near blood vessels (Figure 4b). Exposed animals (group B) had a kidney of regressive cancerous cells. Metastasized cells were scarsly infiltrated between tubular structures (Figure 4c).

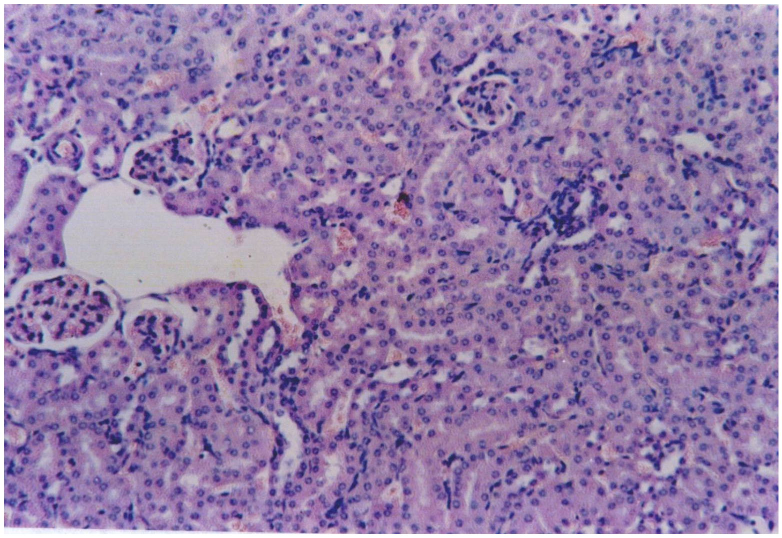

Figure 4c. A histological section of the kidney of the animals from group B shows a regression of cancerous cells. Metastasized cells were scarsly infiltrated between tubular structures (Magnification x 200).

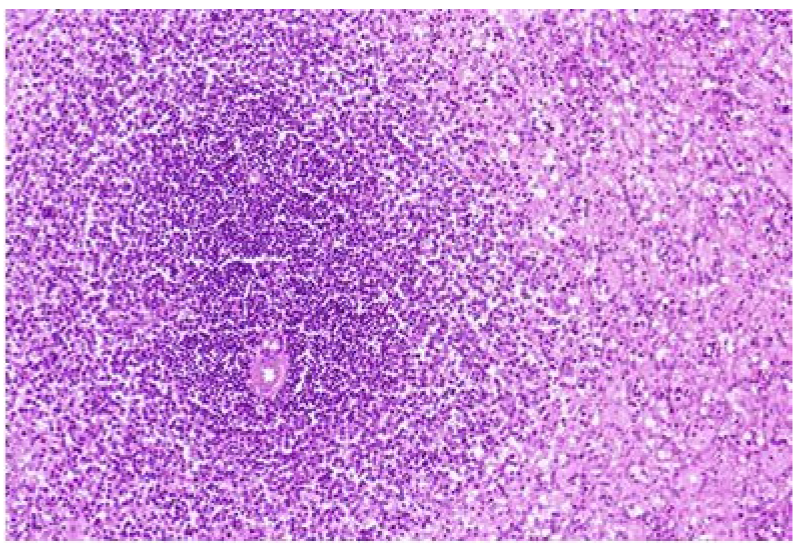

Figure 5a. A photographic cross section for a normal splenic tissue (Magnification x 200).

Spleen

A histological section of normal spleen is shown in Figure 5a. Histological configurations of spleen in group A revealed metastasized malignant cells were equally distributed in splenic tissue. Neoplastic cells remained with the above described malignant criteria (Figure 5b). In contrast, spleen of group B (exposed mice) appeared lacking to secondary infiltrative Ehrlich growth. Well differentiated splenic white and red pulps are noticed (Figure 5c).

DISCUSSION AND CONCLUSION

In the present work the effect of 4.5 Hz square amplitude modulated wave electric field on tumor metastasis and characteristics in the liver, kidney and spleen of mice injected into the left flank with Ehrlich tumor ascites was studied. The results of the dielectric relaxation studies for liver from healthy and metastatic samples indicated that the relative permittivity, conductivity and dielectric loss versus applied electric field frequency have higher values for liver of group A as compared with normal liver tissues

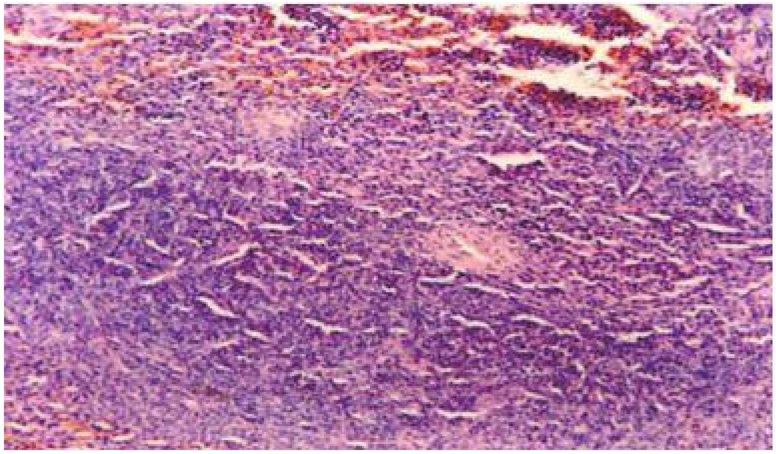

Figure 5b. Histological configurations of spleen in group A revealed metastasized malignant cells were equally distributed in splenic tissue. Neoplastic cells remained with above described malignant criteria (Magnification x 200).

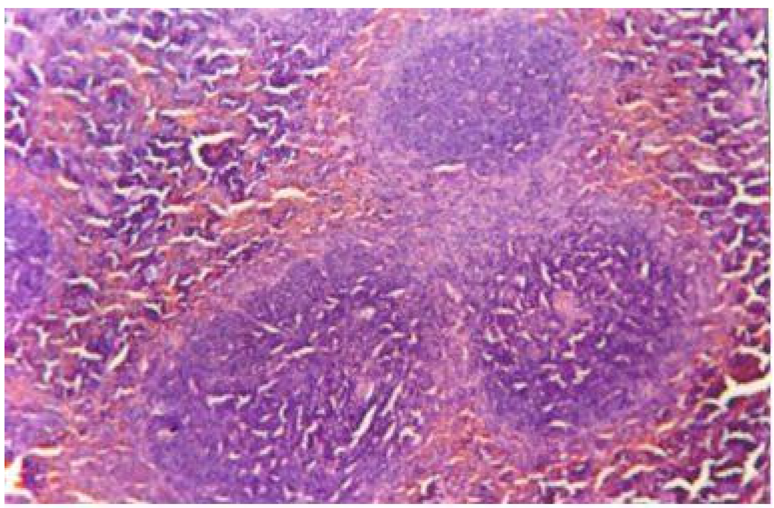

Figure. 5c. A photographic cross section spleen of group B (exposed mice) appeared lacking to secondary infiltrative Ehrlich growth. Well differentiated splenic white and red pulps are noticed (Magnification x 200).

Table 2. Shows the average values of dielectric increment (Δέ), conductivity (σ), relaxation time (τ), in μsec and Cole-Cole parameter (α) for normal liver tissues and the treated groups.

Studied sample	**Dielectric increment** Δέ	**Conductivity (σ) at 10 MHz x 10^{-2}(S/m^{-1})**	**Relaxation time τ (μsec)**	**α**
Normal	3029 ± 140.72	30.008 ± 0.48	0.76 ± 0.028	0.106 ± 0.002
Group A (Unexposed)	4140.4 ± 70.63	38.48 ± 0.47	0.74 ± 0.024	0.11 ± 0.00
GroupB (QAMW exposed)	3738 ± 130.56	35.9± 2.31	0.696 ± 0.023	0.1 ± 0.00

(Figure 2 and Table 2) .These findings are expected since the tumor tissues have significant higher water content than homologous normal tissue.(Surowiec et al., 1988). It is well known that tumor cells have higher surface charges on the cellular membrane than normal cells, so it can be expected that higher counter ion molecules will be attracted to the tumor cell membrane and hence the tumor cell will have higher electrical conductivity than normal tissue. Smith et al. (1986) observed that the conductivity of implanted liver tumor at audio frequency (20 Hz to 20 kHz) is 7 to 10 times higher than normal surrounding tissue. The results in this work indicated that the values of conductivity, dielectric loss and permittivity decreased for treated metastasis liver by 4.5 Hz QAMW waves (group B). These results indicate that the repair mechanism began to be active to have properties similar to healthy tissue. These findings are supported by the histological sections (Figure 3) and are in agreement with the work of Blank who reported, that weak electromagnetic field (EMF) can control and amplify biological processes through their effects on charge distribution (Blank et al., 2008).

The mechanism of interaction of these electromagnetic fields with the tumor cells at this frequency may be the resonance destructive interference with the electric impulses generated from ionic motions in tumor cell division resulting in tumor growth inhibition (Fadel et al., 2005).

To stop uncontrollable cell divisions which lead to metastasis, two processes should be carried out. The first is to enhance the activity of the tumor suppressor genes which discourage cell growth .The second process is the rechange of the oncogenes to proto-oncogenes to promote normal cell growth and function (Vogelstein and Kinzler, 2004).One may add here that there are several parameters that can affect cell division which include DNA properties, cell membrane and intercellular contents. Changes in the properties of one or more of these parameters can enhance or inhibit cellular division. From the dielectric relaxation results, the loss of the surface electrostatic charges upon the cells can be due to changes of the cellular membrane permeability and hence the ionic pump through the membrane. However, still extensive research work is needed to be done in order to give full and solid explanation for the interaction mechanisms of the 4.5 Hz QAMW with the biological system involved in the inhibition of the tumor growth (primary site) and metastasis formation (secondary sites) in the liver, kidney and spleen.

Moreover, application of the 4.5 QAMW on the whole body will cause induced ionic currents and potentials which may result in the prevention of any further mutation in proto-oncogenes to be oncogenes . This analysis is supported by the histological studies for liver, kidney and spleen tissues in group B, where there was an evident regression in tumor large nuclei. Low grade metastatic malignant epithelial cells were arranged in masses and normal healthy tissues predominated in the investigated sections (Figures 3c, 4c and 5c).

From our previous findings by Fadel et al. (2005), and the present results one may conclude that 4.5 Hz QAMW waves are very promising for the treatment of tumors either in primary site state and/or as a preventive agent for metastasis formation in the secondary site (liver, kidney and spleen). However there is still much work to be done to render this technique clinically applicable.

REFERENCES

Blank M (2008). “Protein and DNA reactions stimulated by electromagnetic fields”. Electromagn. Boil. Med., 27(1): 3-23

Cameron IL, Sun LZ, Short N, Hardman WE, Williams CD (2005). "Therapeutic Electromagnetic Field (TEMF) and gamma irradiation on human breast cancer xenograft growth, angiogenesis and metastasis". Cancer Cell Int., 26(5): 23.

Ciria HC, Quevedo MS, Cabrales LB, Bruzon RP, Salas MF, Pena OG, Gonzalez TR, Lopez DS, Flores JM (2004). "Antitumor effectiveness of different amounts of electrical charge in Ehrlich and fibrosarcoma Sa-37 tumors". BMC-Cancer. 2004 Nov 26, 4(1): 87.

Fadel MA, Reem HE, Amany A, Ibrahim F (2005). "Control of Ehrlich tumor growth by electromagnetic waves at resonance frequency in vivo", Electromagn. Biol. Med., 24: 9-12.

Richard N, Uwe P, Xinhua C, Wentia F, James SR, Stephen JB, Juergen FK, Karl HS (2006). " Nanosecond pulsed electric fields cause melanomas to self-destruct". Biochem. Biophys. Res. Commun., 5,343(2): 351-360.

Robertson G, Wemyss H, Dennison A, Hall P, Baxter P, Maddern G (1998). "Experimental study of electrolysis-induced hepatic necrosis". Br J Surg., Sep 1998; 85(9): 1212-1216.

Smith SR, Foster KR, Wolf JL (1986). "Dielectric properties of VX-2 carcinoma vs. normal liver tissue", IEEE Trans. Biomed. Eng., BME-33, 522.

Snedecor GW, Coechran WG (1967). Statistical methods, 6th ed., Iowa State University Press, Ames, pp. 91-135.

Surowiec AJ, Stuchly SS, Barr JR, Swarup A (1988). "Dielectric properties of breast carcinoma and the surrounding tissue", IEEE Trans. Biomed. Eng., BME-35, 257.

Traitcheva N, Angelova P, Radeva M, Berg H (2003). "ELF fields and photooxidation yielding lethal effects on cancer cells". Bioelectromagnetics, 24(2): 148-50.

Turler A, Schaefer H, Schaefer N, Maintz D, Wagner M, Qiao J, Hoelscher A (2000). "Local treatment of hepatic metastases with low-level direct electric current: experimental results". Scand. J. Gastroenterol., Mar 2000; 35(3): 322-328.

Turler A, Schafer H, Schafer N, Fischbach R, Wagner M, Qiao JC, Holscher AH (1998). "Pilot study of effectiveness of electrotherapy in the experimental liver metastasis model", Langenbecks Arch Chir Suppl Kongressbd.1998; 115(1): 611-614.

Venook AP (1994). "Hepatocellular carcinoma: Too many options?", J. Clin. Oncol., 12: 1323.

Vogelstein B, Kinzler KW (2004). "Cancer genes and the pathways they control" .Nat. Med., p. 10.